REMARKS

The Examiner is thanked for having withdrawn the prior rejections.

Claim 1 has been amended to recite "[a] recombinant microorganism of *Escherichia coli* being capable of producing vitamin B_6, wherein said microorganism carries extra nucleic acids encoding **[[an]]** a synergistic enzyme combination selected from the group consisting of:

i) erythrose 4-phosphate dehydrogenase encoded by a polynucleotide obtained from *E. coli* chromosomal DNA by PCR using primers of SEQ ID NO: 1 and SEQ ID NO: 2 and 1-deoxy-D-xylulose 5-phosphate synthase encoded by a polynucleotide obtained from *E. coli* chromosomal DNA by PCR using primers of SEQ ID NO: 5 and SEQ ID NO: 6;

ii) erythrose 4-phosphate dehydrogenase encoded by a polynucleotide obtained from *E. coli* chromosomal DNA by PCR using primers of SEQ ID NO: 1 and SEQ ID NO: 2 and pyridoxol 5'-phosphate synthase encoded by a polynucleotide obtained from *E. coli* chromosomal DNA by PCR using primers of SEQ ID NO: 9 and SEQ ID NO: 10; and

iii) erythrose 4-phosphate dehydrogenase encoded by a polynucleotide obtained from *E. coli* chromosomal DNA by PCR using primers of SEQ ID NO: 1 and SEQ ID NO: 2, 1-deoxy-D-xylulose 5-phosphate synthase encoded by a polynucleotide

obtained from *E. coli* chromosomal DNA by PCR using primers of SEQ ID NO: 5 and SEQ ID NO: 6 and pyridoxol 5'-phosphate synthase encoded by a polynucleotide obtained from *E. coli* chromosomal DNA by PCR using primers of SEQ ID NO: 9 and SEQ ID NO: 10.

Support for the amendment is found in the Specification at, for Example, page 9, line 7 to page 10, line 3 (second paragraph of Example 11 and Table I). The amendment adding complete Markush language places the claim in proper form and does not alter the scope of the claim. No new matter has been added.

Obviousness Rejections

A. Zhao in view of Sprenger and Laber

Claim 1 was rejected under 35 U.S.C. § 103(a) as obvious over Zhao et al. (J Bacteriol. 1995; 177(10):2804-12) ("Zhao") in view of "the combined teachings of" Sprenger et al. (Proc Natl Acad Sci USA. 1997; 94(24):12857-62) ("Sprenger") and Laber et al. (FEBS Lett. 1999; 449(1):45-8) ("Laber"). (Paper No. 20080503 at 3.)

Zhao discloses "the purification and enzymological characterization of the *gapB*-encoded dehydrogenase. [The] results confirm the hypothesis that the *gapB*-encoded enzyme is an [E4P dehydrogenase (E4PDH)], rather than a second [glyceraldehyde 3-phosphate dehydrogenase (GA3PDH)]." (Page 2804, right Col., lines 19-22.) Zhao also discloses that "results [are presented that are] consistent with the

idea that this E4PDH plays a role in the de novo biosynthesis of the [pyridoxal 5'-phosphate (PLP)] coenzyme." (Id. at lines 22-24.)

Sprenger discloses that "[i]n *Escherichia coli*, 1-deoxy-D-xylulose (or its 5-phosphate, DXP) is the biosynthetic precursor to isopentenyl diphosphate [citations omitted], thiamin, and pyridoxol [citations omitted]." (Abstract, lines 1-6.) Sprenger also discloses that "evidence [is provided] that in *E. coli* synthesis of DXP from the precursors pyruvate and glyceraldehyde 3-phosphate is performed by a thiamin diphosphate-dependent enzyme, DXP synthase." (Page 12861, left Col., second line from the bottom to right Col., line 2.) Sprenger further discloses that the dxs gene was cloned and overexpressed, and "[t]he reaction catalyzed by DXP synthase yielded exclusively DXP...". (Abstract, lines 12-15.)

Laber discloses that "[i]n *Escherichia coli* the coenzyme pyridoxal 5'-phosphate (PLP) is synthesized de novo by a pathway that is thought to involve the condensation of 4-(phosphohydroxy)-L-threonine and 1-deoxy–D-xylulose, catalyzed by the enzymes PdxA and PdxJ, to form either pyridoxine (vitamin B_6) or pyridoxine 5'-phosphate (PNP)." (Abstract, lines 1-6.) Laber also discloses that "[t]he exact [role], however, played by the ... *pdxJ* gene [product], ... PdxJ, [has] long been undetermined... [W]e present evidence that purified PdxJ catalyses the in vitro formation of PNP in the presence of purified PdxA, HTP, NAD and 1-deoxy-D-xylulose-5-phosphate, but not 1-deoxy-D-xylulose." (Page 45, left Col., fifth line from the bottom to right Col., line 3.)

In making the rejection, the Examiner asserted that Zhao discloses "a recombinant *Escherichia coli* capable of producing vitamin B_6 comprising extra nucleic

acids from *Escherichia coli* (*epd* gene) encoding erythrose 4-phosphate dehydrogenase, which is expected to be amplified using the recited PCR primers of SEQ ID NOs: 1 and 2. See entire publication, especially pages 2804-2810, Figs. 1 and 2, and Tables 1-3." (Paper No. 20080503 at 3.)

The Examiner acknowledged, however, that "[t]he teachings of Zhao et al. differ from the claims in that the recombinant *Escherichia coli* does not carry extra nucleic acids encoding 1-deoxy-D-xylulose 5-phosphate synthase and pyridoxol 5'-phosphate synthase." (Id.)

The Examiner asserted that Sprenger discloses the "nucleic acid from *Escherichia coli* encoding 1-deoxy-D-xylulose 5-phosphate synthase, which is required for the formation of the 1-deoxy-D-xylulose 5-phosphate precursor to vitamin B_6 and is expected to be amplified using the recited PCR primers of SEQ ID NOs: 5 and 6. See entire publication, especially pages 12857-12861 and Figs. 1-4." (Id.)

The Examiner asserted that Laber discloses that "the nucleic acid from *Escherichia coli* encoding pyridoxol 5'-phosphate synthase (PdxJ protein), which in combination with 4-(phosphohydroxy-L-threonine dehydrogenase (PdxA protein) catalyzes the formation of vitamin B_6 and is expected to be amplified using the recited PCR primers of SEQ ID NOs: 9 and 10. See entire publication, especially pages 45-47." (Id.)

The Examiner concluded that "it would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the recombinant *Escherichia coli* of Zhao et al. such that the *E. coli* nucleic acid encoding 1-deoxy-D-xylulose 5-phosphate synthase taught by Sprenger et al. and the *E. coli* nucleic acid

encoding pyridoxol 5'-phosphate synthase taught by Laber et al. are transformed and overexpressed in the recombinant *Escherichia coli* of Zhao et al. One of ordinary skill in the art at the time the invention was made would have been motivated to do this in order to have a recombinant *Escherichia coli* that can ***overproduce*** Vitamin B_6 due to the overexpressed and overproduced enzymes within the modified recombinant *Escherichia coli* of Zhao et al. One of ordinary skill in the art at the time the invention was made would have a reasonable expectation of success because the art of molecular biology and recombinant manipulations of *E. coli* host cells are well known and developed." (Id. at 3-4.) (emphasis added).

The rejection is respectfully traversed.

Claim 1 has been amended to recite that the "microorganism carries extra nucleic acids encoding a synergistic combination selected from the group consisting of...".

It is well settled the Examiner bears the burden to set forth a *prima facie* case of unpatentability. *In re Glaug*, 62 USPQ2d 1151, 1152 (Fed. Cir. 2002); *In re Oetiker*, 24 USPQ2d 1443, 1444 (Fed. Cir. 1992); and *In re Piasecki*, 223 USPQ 785, 788 (Fed. Cir. 1984). If the PTO fails to meet its burden, then the applicant is entitled to a patent. *In re Glaug*, 62 USPQ2d at 1152.

When patentability turns on the question of obviousness, as here, the search for and analysis of the prior art by the PTO should include evidence relevant to the finding of whether there is a teaching, motivation, or suggestion to select and modify the document(s) relied on by the Examiner as evidence of obviousness. *KSR Int'l Co. v. Teleflex Inc.*, 127 S.Ct. 1727, 1731-32 (2007) (the obviousness "***analysis***

should be made explicit" and the teaching-suggestion-motivation test is "***a helpful insight***" for determining obviousness) (emphasis added); *McGinley v. Franklin Sports*, 60 USPQ2d 1001, 1008 (Fed. Cir. 2001). Moreover, the factual inquiry whether to modify document(s) must be thorough and searching. And, as is well settled, the teaching, motivation, or suggestion test "***must be based on objective evidence of record***." *In re Lee*, 61 USPQ2d 1430, 1433 (Fed. Cir. 2002) (emphasis added). *See also Examination Guidelines for Determining Obviousness*, 72 Fed. Reg. 57526, 57528 (October 10, 2007) ("The key to supporting any rejection under 35 USC § 103 is the clear articulation of the reason(s) why the claimed invention would have been obvious.").

Respectfully, we submit that the rejection is devoid of a proper § 103 analysis in support of the proposed modification. All that is there are conclusory statements such as the assertion that "it would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the recombinant *Escherichia coli* of Zhao et al. such that the *E. coli* nucleic acid encoding 1-deoxy-D-xylulose 5-phosphate synthase taught by Sprenger et al. and the *E. coli* nucleic acid encoding pyridoxol 5'-phosphate synthase taught by Laber et al. are transformed and overexpressed in the recombinant *Escherichia coli* of Zhao et al." and that "[o]ne of ordinary skill in the art at the time the invention was made would have been motivated to do this in order to have a recombinant *Escherichia coli* that can ***overproduce*** Vitamin B_6 due to the overexpressed and overproduced enzymes within the modified recombinant *Escherichia coli* of Zhao et al." (Paper No. 20080503 at 3-4.) (emphasis added).

Here, what the rejection should have done, but did not, was to explain on the record ***why*** one skilled in this art would modify the disclosure of Zhao, Sprenger and/or Laber in the manner proposed by the Examiner to arrive at the claimed recombinant microorganism in which the microorganism carries extra nucleic acids encoding "a synergistic combination" selected from the recited combinations. As is well settled, an Examiner cannot establish obviousness by locating documents which describe various aspects of a patent applicant's invention without also providing evidence of the motivating force which would impel one skilled in the art to do what the patent applicant has done. *Takeda Chem. Indus., Ltd v. Alphapharm Pty., Ltd.*, 492 F.3d 1350, 1357 (Fed. Cir. June 28, 2007) (citing *KSR*) (indicating that "it remains necessary to identify ***some reason*** that would have led a chemist to modify a known compound in a particular manner to establish prima facie obviousness of a new claimed compound") (emphasis added); *Ex parte Levengood*, 28 USPQ2d 1300, 1301-02 (BPAI 1993). But this is precisely what the Examiner has done here. Thus, the rejection is legally deficient and should be withdrawn for this reason alone.

Beyond looking at the cited documents to determine if any of them suggests doing what the inventors have done, one must also consider if the documents provide the required expectation of succeeding in that endeavor. See *In re Dow Chem. Co. v. American Cyanamid Co.*, 5 USPQ2d 1529, 1531 (Fed. Cir. 1988) ("Both the suggestion and the expectation of success must be founded in the prior art, not in applicants' disclosure.") "Obviousness does not require absolute predictability, but a reasonable expectation of success is necessary." *In re Clinton*, 188 USPQ 365, 367 (CCPA 1976). Following the Supreme Court's decision in *KSR Int'l Co. v. Teleflex Inc.*,

127 S.Ct. 1727 (2007), the *U.S. Patent and Trademark Office Examination Guidelines for Determining Obviousness* were issued which provide the following guidance to Examiners at page 57527: "In short, the focus when making a determination of obviousness should be on what a person of ordinary skill in the pertinent art would have known at the time of the invention, and on what such a person would have reasonably expected to have been able to do in view of that knowledge". However, no such motivation or expectation of success can be found in the cited documents.

It is submitted that neither Zhao, Sprenger, nor Laber, alone or in any combination, teach, suggest, or provide motivation to achieve the claimed recombinant microorganism, wherein the microorganism carries extra nucleic acids encoding a ***synergistic enzyme combination*** as selected from combinations i), ii) or iii) as recited in claim 1. Furthermore, neither Zhao, Sprenger, nor Laber, alone or in any combination, provide an expectation of success of achieving the claimed recombinant microorganism, wherein the microorganism carries extra nucleic acids encoding a ***synergistic enzyme combination*** as selected from combinations i), ii) or iii) as recited in claim 1.

None of the cited documents, Zhao, Sprenger, or Laber, disclose the combinations as claimed. Zhao and Sprenger each only disclose the effect on vitamin B_6 production of a single enzyme. Laber, to the extent that it may disclose a combination, discloses only the "overproduction" and purification of PdxA and PdxJ polypeptides by amplification of pdxA and pdxJ. (Page 45, right Col., section 2.2.) There is no suggestion to combine any of the disclosed nucleic acids of the cited documents. Furthermore, the combination pdxA and pdxJ of Laber in no way leads one

to the combinations as presently claimed. And, even assuming *arguendo* that combining the nucleic acids is suggested, there is still no disclosure or suggestion that making such a combination would result in synergy as claimed.

For example, as disclosed in Example 11 of the present application, only the claimed combinations listed in Table I resulted in a synergistic enzyme combination. The recombinant *E. coli* strains shown in Table 1 were used for fermentative production of vitamin B_6. The amount of vitamin B_6 was determined for the host strain, *E. coli* AT1024, and for the host strain microorganism in which one of the enzymes E4P dehydrogenase, PNP synthase or DXP synthase had been overexpressed (using the single extra plasmid pKK-epd, pKK-pdxJ, or pKK-dxs, respectively), and for the microorganism in which a combination of PNP synthase and DXP synthase had been overexpressed (using the extra plasmid combination pKK-pdkJ/pVK-dxs). (Example 11, Table 1.) The amount of vitamin B_6 obtained was disclosed as being "7.1-fold, 2.22-fold, and 1.95-fold higher [for the recombinant strains harboring the pKK-epd plasmid, pKK-pdxJ plasmid, and the pKK-dxs plasmid, respectively] than vitamin B_6 accumulation in the host strain, *E. coli* AT1024." (Page 9, lines 9-12.) The amount of vitamin B_6 for the plasmid combination pKK-pdkJ/pVK-dxs was 2.7-fold higher as compared to vitamin B_6 accumulation in the host strain. (Table I.)

In using the plasmid combinations pKK-edp and pVK-dxs; and pKK-epd and pVK-pdxJ, on the other hand, the amount of vitamin B_6 was "synergistically" increased with a 24.6-fold and 28.9-fold increase, respectively, over the accumulation in the host strain. (Table I.) As is disclosed in the Specification, "among recombinant *E. coli* strains carrying a combination of two extra plasmids, only the combination which

contains epd[, i.e., includes the enzyme E4P dehydrogenase,] showed marked increase in the amount of vitamin B_6, suggesting the synergistic effects of the [combinations] containing epd. This markedly improved effect was not shown, however, with the combination of DXP and PNP synthase overexpression (using pKK-pdkJ/pVK-dxs as noted above). Further, it is noted that the combination which contains the three extra plasmids, pKK-edp/pVK-pdxJ/pSTV-dxs, shows a 39.3-fold improvement, a combination demonstrating an even more striking improvement. (Table I.)

One skilled in the art would not have understood Zhao, Sprenger, and/or Laber to suggest any synergistic combinations, nonetheless the ones recited in claim 1. Moreover, as the synergistic results obtained implicate combination with epd as noted above, the disclosed amplification of pdxA and pdxJ of Laber which does not include epd tends to lead one away from the claimed recombinant microorganism. As such, the claimed invention provides unexpected synergistic results regarding the recombinant microorganism of claim 1.

Furthermore, the claimed recombinant microorganism carries extra nucleic acids encoding a synergistic enzyme combination from among the recited, select group of combinations. It could not have been expected by one of ordinary skill in the art that the recited combinations including the overexpression of E4P dehydrogenase would lead to a synergistic effect in vitamin B_6 production, whereas a combination of e.g., the genes encoding DXP and PNP synthase (pdxJ/pVK-dxs), did not show as great an improved effect as compared to the combinations containing epd. (Table I.)

We also note that the Examiner focused his analysis on "overproduction" (Paper No. 20080503 at 3). But, as noted above, the claims recite a "synergistic" enzyme combination. It is respectfully submitted that simply overproducing vitamin B_6 is not the same as obtaining a "synergistic enzyme combination." Thus, the rejection is based on a misunderstanding of the claimed subject matter and should be withdrawn for this reason as well.

It is respectfully submitted that the rejection has been rendered moot. Reconsideration and withdrawal of the rejection are requested.

B. Zhao in view of Sprenger, Laber, and Yang

Claims 3 and 6-8 were rejected under 35 U.S.C. § 103(a) as being unpatentable over Zhao in view of "the combined teachings of" Sprenger and Laber as applied to claim 1 above, and further in view of Yang et al. (J Bacteriol. 1998; 180(16):4294-9) ("Yang"). (Paper No. 20080503 at 4.)

Zhao, Sprenger, and Laber are summarized in section A above.

Yang discloses that "*epd (gapB)* mutants lacking an erythrose 4-phosphate (E4P) dehydrogenase are impaired for growth on some media and contain less pyridoxal 5'-phosphate (PLP) and pyridoxamine 5'-phosphate (PMP) than their epd^+ parent." (Abstract, lines 1-3.) Yang further discloses that the "results implicate the GapA and Epd dehydrogenases in de novo PLP and PMP coenzyme biosynthesis...". (Abstract, lines 4-5.)

In making the rejection, the Examiner incorporated the assertions made regarding Zhao, Sprenger, and Laber from the first obviousness rejection which is addressed in section A above.

Further with regard to the present rejection, the Examiner asserted that "Yang et al. teach a process for preparing vitamin B_6 comprising culturing recombinant *Escherichia coli* strains having the *epd* gene encoding erythrose 4-phosphate dehydrogenase in LB medium (fermentation broth) containing 1% glycerol and 1% succinate at 37°C for about 24 hours. Yang et al. further teach HPLC chromatography to identify B_6 vitamers. See entire publication especially pages 4294-4298, Figs. 1-3, and Tables 1-3." (Paper No. 20080503 at 4.)

The Examiner concluded that "it would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Yang et al. such that the modified recombinant *Escherichia coli* of Zhao et al. is used in the process for preparing vitamin B_6 taught by Yang et al. and the produced vitamin B_6 separated from the fermentation broth. One of ordinary skill in the art at the time the invention was made would have been motivated to do this in order to have a fermentation method that will allow production of large amounts of vitamin B_6. Furthermore, it is within the preview of one of ordinary skill in the art at the time the invention was made to use and optimize the recited temperature, pH, nutrients, carbon source, nitrogen source, inorganic salts, and culturing conditions in order to facilitate optimal production of vitamin B_6." (Id.)

The rejection is traversed.

As noted above, claim 1 has been amended to recite that the "microorganism carries extra nucleic acids encoding a synergistic combination selected from the group consisting of...". We note that claim 3 recites "a process for preparing vitamin B_6 comprising the steps of i) culturing ***the recombinant microorganism of claim 1*** in a fermentation broth; and ii) separating the resulting vitamin B_6 from the fermentation broth." Claims 6-8 depend from claim 3.

We incorporate all arguments presented above in section A in response to the rejection of claim 1 here as though presented in full with regard to the rejection of claims 3 and 6-8. We note that for the reasons set forth below, Yang fails to fill these gaps.

Indeed, Yang provides no teaching or suggestion that addresses the deficiencies in the Examiner's combination of Zhao, Sprenger, and/or Leber, nor that would provide a teaching or suggestion to combine Yang with the cited documents in the manner suggested by the Examiner.

It is respectfully submitted that the process for preparing vitamin B_6 of claim 3 in which a recombinant microorganism that carries extra nucleic acids encoding a synergistic enzyme combination (as recited in claim 1) is not taught or suggested by Zhao, Sprenger, Leber and/or Yang, alone or in any combination. One skilled in the art would not have expected success in achieving the claimed process that uses a recombinant microorganism to produce synergistic amounts of vitamin B_6. Nor would one skilled in the art have known to select the combinations of extra nucleic acids as recited in view of any or all of the cited documents, to achieve the synergistic enzyme combination of the claimed process.

It is submitted that the present rejection has been rendered moot. Reconsideration and withdrawal of the rejection are requested.

Accordingly, for the reasons set forth above, entry of the amendments, withdrawal of the rejections, and allowance of the claims are respectfully requested. If the Examiner has any questions regarding this paper, please contact the undersigned.

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Mail Stop Amendment, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on October 7, 2008.

Eileen M. Ebel, Reg. No. 37,316

Respectfully submitted,

By: _____

Eileen M. Ebel
Registration No. 37,316
BRYAN CAVE LLP
1290 Avenue of the Americas
New York, NY 10104-3300
Phone: (212) 541-2000
Fax: (212) 541-4630